

Larval Development of the Tropical Deep-Sea Echinoid *Aspidodiadema jacobyi*: Phylogenetic Implications

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Abstract. The complete larval development of an echinoid in the family Aspidodiadematidae is described for the first time from *in vitro* cultures of *Aspidodiadema jacobyi*, a bathyal species from the Bahamian Slope. Over a period of 5 months, embryos grew from small (98- μm) eggs to very large (3071- μm) and complex planktotrophic echinopluteus larvae. The fully developed larva has five pairs of red-pigmented arms (preoral, anterolateral, postoral, posterodorsal, and posterolateral); fenestrated triangular plates at the bases of fenestrated postoral and posterodorsal arms; a complex dorsal arch; posterodorsal vibratile lobes; a ring of cilia around the region of the preoral and anterolateral arms; and a long, unpaired posterior process containing a fenestrated rod. The presence of a posterior process and posterodorsal arms makes the larva of *Aspidodiadema jacobyi* much more similar to larvae of irregular urchins in the order Spatangoida than to other families of the order Diadematoida, to which the family is normally assigned. This unexpected larval form lends support to a recommendation that the Aspidodiadematidae should be either elevated to ordinal status as a sister group of the order Diadematoida, or split off as a sister group of the other families within the order. In either case, if we accept the parsimonious hypothesis that the aboral process and posterodorsal arms were derived only once in the evolutionary history of euechinoids, then the larval data suggest that the Aspidodiadematidae may be very near the node where the irregular and regular euechinoids first diverged.

Introduction

Larval developmental mode has been inferred from egg size for a large number of echinoderm species from the deep sea, but only a few of these have been cultured into the early larval stages (Prouho, 1888; Mortensen, 1921; Young and Cameron, 1989; Young *et al.*, 1989), and no complete ontogenetic sequence of larval development has been published for any deep-sea invertebrate. One of the species whose early stages have been described (Young *et al.*, 1989) is *Aspidodiadema jacobyi*, a small-bodied sea urchin with long flexible spines that lives at bathyal depths in the tropical eastern Atlantic (Fig. 1). The early development of *A. jacobyi* is unusual in that its small eggs (94–100 μm) contain sufficient yolk reserves to support a prefeeding developmental period that extends well into the larval stage (Young *et al.*, 1989), a feature that might permit larvae to move long distances, either vertically or horizontally, before feeding becomes necessary. In this paper, we describe the complete larval development of *A. jacobyi*, document unexpected morphological traits in the late-stage larvae, and discuss how these unexpected traits could help justify a realignment of the currently accepted ordinal-level taxonomy of the Euechinoidea.

The echinoid family Aspidodiadematidae contains only two genera, *Aspidodiadema* and *Plesiodiadema*, all species of which are found at bathyal and abyssal depths (Hyman, 1955), many on island slopes in tropical seas. On the basis of adult characters, notably aulodont teeth and hollow spines, Mortensen (1927) placed the family in the order Diadematoida, suborder Aulodonta, in the company of the Echinothuriidae, the Pedinidae, and the Diadematidae. Recent authors (*e.g.*, Smith, 1984) have elevated the echinothuriids and pedinids to ordinal level, placing three fam-

Received 8 December 1999; accepted 1 February 2000.

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Figure 1. Adult *Aspidodiadema jacobyi* from the Bahaman Slope, showing long flexible spines. (Photo by L. F. Braithwaite).

ilies in the order Diadematoidea: the Aspidodiadematidae, the Diademmatidae, and the Lissodiadematidae. Larvae of the Lissodiadematidae remain unknown (Emlet, 1988a; Pearse and Cameron, 1991). Emlet (1988a) has argued on the basis of larval form that the Micropygidae, which have also been raised to ordinal status by Jensen (1981), should also be included as a family in the Diadematoidea. Although it seems clear that the aspidodiadematids are closely related to the diademmatoids, the relationships among orders and families near the base of the euechinoid tree remain obscure. This portion of the tree remains inadequately resolved because a thorough cladistic analysis of extant species has not been undertaken (R. Mooi, California Academy of Sciences, San Francisco, pers. comm.), and also because fossil material from the relevant time periods contains too few meaningful characters for construction of a robust tree (A. B. Smith, The Natural History Museum, London, pers. comm.). Fossil aspidodiadematids closely resembling modern urchins in the genus *Plesiadiadema* have been described from the upper Jurassic clays of England and France (Smith, 1995), indicating that the family differentiated more than 200 million years ago.

By analyzing the crystalline axes of echinoid apical plates (Raup, 1965), Emlet (1985, 1988a) was able to infer certain details of larval form for a number of rare species with unknown larvae. On the basis of this indirect evidence, he predicted that all members of the genera *Aspidodiadema* and *Plesiadiadema* should have larvae with both preoral and posterodorsal arms (Emlet, 1988a). This prediction is in stark contrast to the 2-arm larvae with widely spread arms (the "*Echinopluteus transversus*" of Mortensen, 1921) found in all members of the closely related families Diademmatidae and Micropygidae. By assuming that the 2-arm larval form with widely spread arms is synapomorphic, Emlet (1988a) argues parsimoniously that the Aspidodiadematidae must be a sister group to the Diadematoidea, having branched off the major clade of regular euechinoids earlier than the diademmatids or micropygids. Indeed, he recommends that the Aspidodiadematidae should be removed from the Diademmatoids and elevated to the rank of order. In

the present paper, we confirm Emlet's prediction concerning the presence of posterodorsal arms in the Aspidodiadematidae and document other larval characters supporting the idea that this family of urchins may have originated near the node where the regular and irregular urchins diverged.

Materials and Methods

Adult *Aspidodiadema jacobyi* were collected by suction using Johnson-Sea-Link submersibles between depths of 500 and 750 m at various sites in the Northern Bahamas (Young, 1992). They were transported to the surface in closed acrylic containers of seawater and transferred to 14°C incubators within minutes of arriving at the surface. Although we collected this species during the spring months of every year from 1985 through 1999, we were able to induce spawning only twice, once in 1987 (Young *et al.*, 1989) and once on 11 April 1994. Spawning was induced by intracoelomic injection of 0.55 M KCl. In the 1994 spawning, we injected 28 individuals; of these, 13 males and 2 females released gametes. Cultures were established by mixing the eggs of a single female with active sperm from 6 males in a small bowl of seawater. The other female was not used because she released very few oocytes, many of which appeared irregular. Zygotes were split among five 1-l jars of 0.45- μ m filtered seawater, at an initial density of 800 embryos per liter. After larvae had developed complete guts, they were fed with a mixture of the algae *Isochrysis galbana*, *Thalassiosira weissflogii*, and *Dunaliella tertiolecta*. At the outset, we used an algal concentration of 1000–2000 cells/ml, but this was increased gradually as the larvae grew, up to a maximum of 5000 cells/ml, 49 days after fertilization and thereafter. These algal concentrations were chosen because similar concentrations promote optimal larval growth in some shallow-water echinoderm larvae (Fenaux *et al.*, 1985; George, 1990; George *et al.*, 1990). Larvae were reared in dark incubators except during water changes, when they were exposed briefly to the fluorescent overhead lights. To simulate temperature changes that bathyal larvae would encounter if they undertook ontogenetic vertical migration (Young *et al.*, 1996a), we increased the culture temperature from 14°C to 16°C 14 days after fertilization, reduced it to 14°C 66 days after fertilization, then finally to 13°C (the temperature near the bottom of the adult depth range) 77 days after fertilization. Initially, seawater was changed and the larvae were fed every 2 days. This interval was increased to 4 days beginning 28 days after fertilization.

Each time water was changed, we examined the larvae for the appearance of any new arms or other structures and measured the lengths of the postoral, somatic, posterodorsal, and posterolateral rods. Because there were 2-day or 4-day intervals between observations, the times for reported morphological changes (Table 1) should be regarded as only

Table 1

Developmental timetable for *Aspidodiadema jacobyi*, showing the presence (x) and absence (—) of larval arms and other important structures on days when cultures were examined

Days after fertilization	Larval arms					Other structures					
	Postoral	Anterolateral	Posterodorsal	Preoral	Posterolateral	Mouth	Posterior process	Ciliated ring	Posterodorsal lobes	Echinus rudiment	Podia
6	x	—	—	—	—	—	—	—	—	—	—
9	x	x	—	—	—	—	—	—	—	—	—
11	x	x	—	—	—	x	—	—	—	—	—
15	x	x	—	—	—	x	—	—	—	—	—
25	x	x	x	—	—	x	—	—	—	—	—
28	x	x	x	—	—	x	—	—	—	—	—
32	x	x	x	—	—	x	x	—	—	—	—
34	x	x	x	x	—	x	x	—	—	—	—
35	x	x	x	x	—	x	x	—	—	—	—
39	x	x	x	x	—	x	x	—	—	—	—
42	x	x	x	x	x	x	x	—	—	—	—
46	x	x	x	x	x	x	x	—	—	—	—
49	—	—	x	x	x	x	x	x	—	—	—
53	—	—	x	x	x	x	x	x	—	—	—
56	—	—	x	x	x	x	x	x	x	—	—
62	—	—	x	x	x	x	x	x	x	—	—
75	—	—	x	x	x	x	x	x	x	x	—
116	—	—	x	x	x	x	x	x	x	x	x
120	—	—	x	x	x	x	x	x	x	x	x

Larvae were reared at 14–16°C (see text for details). Because cultures were not examined every day, the times do not accurately represent the first occurrences of larval stages.

approximate markers for the initiation of larval stages. During the early larval stages, total larval length was defined as the linear measurement from the tip of the postoral rod to the point where the two somatic rods meet mid-sagittally on the posterior end of the larva. In the later stages, total larval length was defined as the linear measurement from the tip of the postoral rod to the posterior end of the unpaired aboral process. During the early stages, we generally made all measurements on at least 10 larvae. After the 46th day, when relatively few larvae remained in the cultures, we were concerned about stressing the remaining larvae, so we measured only the most advanced larva at each sampling period. Terminology used for larval arms and spicules is from Mortensen (1921).

Results

Larval growth and form

The early developmental stages of *Aspidodiadema jacobyi* have been described by Young *et al.* (1989), so the present study characterizes only the echinopluteus larval stages. The developmental timetable is summarized in Table 1 and linear growth is plotted in Figure 2. Mortality, size, and stage variability increased over time, but living larvae from all cultures generally appeared healthy, regardless of stage. Decreasing the temperature from 16°C to 13°C

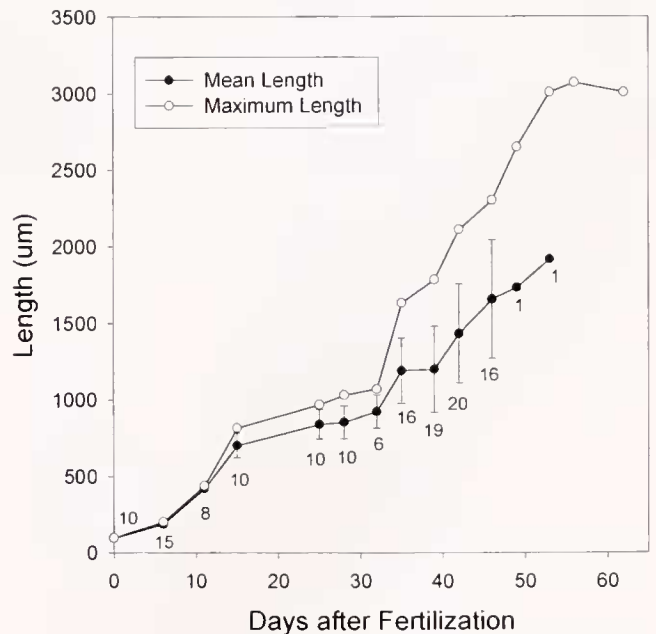


Figure 2. Mean and maximum observed body lengths of *Aspidodiadema jacobyi* larvae during the first 63 days after fertilization. Error bars are standard deviations. Sample size is reported directly below each mean value.

after the 77th day appeared to have a positive effect on larval development: larvae transferred to the lower temperature continued to increase in length and width, whereas larvae that remained at the higher temperature increased in length only. Throughout development, larvae swam with their anterior ends upwards, remaining near the surface during the early larval stages (2- to 6-arm stages) and near the bottom during later larval stages (8- and 10-arm stages).

The 2-arm stage began 6 days after fertilization with the appearance of postoral arms (Fig. 3A). Fenestrated arm rods were visible by cross-polarized light, but opacity of the gut region obscured skeletal details in the posterior end, as previously reported by Young *et al.* (1989). The postoral arms were widely spread, and red pigment spots were scattered throughout the larval arms. Nine days after fertilization, the anterolateral arms appeared, forming the 4-arm echinopluteus. The gut region became progressively less opaque during this period, and the digestive system could be clearly seen (Fig. 3B). Eleven days after fertilization, larvae had grown to more than twice the original length of the early embryos (Fig. 2). At this stage, the anterolateral arms were very small, but the mouth opened and the larvae began to feed. Fifteen days after fertilization, 4-arm larvae with well-developed postoral and anterolateral arms (Fig. 3C) were present in all cultures, and by the 25th day, these larvae were more than 700 μm in length (Fig. 2).

The posterodorsal arms characteristic of the 6-arm stage appeared 25 days after fertilization (Table 1, Fig. 3D) and were fully developed (Fig. 3E) by the 28th day. Some larvae remained in the 6-arm stage for up to 15 days, but small buds of preoral arms were apparent in some individuals as early as the 34th day (Fig. 3E). Red pigment spots initially present throughout the larval arms became increasingly concentrated at the tips after this stage. A gradual change in larval shape became apparent after the 28th day, and 4 days later an unpaired process supported by a fenestrated rod appeared at the posterior end of the larva (Fig. 3F). This posterior process continued to grow throughout the 6-arm stage and subsequent stages until it was as long as the rest of the larval body. At 16°C, the larvae remained in the 6-arm stage for about 10 days (Table 1).

By the 39th day, there was considerable variability among cultures in the relative proportions of individuals in different larval stages. Some culture jars contained only 6-arm larvae with posterior processes, whereas up to 60% of larvae in other cultures had attained an 8-arm stage. During the 8-arm stage and early 10-arm stage (Fig. 3G), the posterodorsal arms and the posterior process were similar in length, about one-third less than the length of the postoral arms. The base of the postoral arms broadened considerably at this stage and measured 122.4 μm in width.

Between the 39th and 42nd day, the buds of the posterolateral arms formed, thereby initiating the 10-arm stage (Fig. 3G). By the 42nd day these arms were fully developed

in some individuals (Fig. 3H, I). Forty-six days after fertilization, however, as many as 80% of the larvae were still at the 8-arm stage. During the 10-arm stage, the posterodorsal and posterolateral arms continued to lengthen and widen, while the anterolateral and preoral arms were generally resorbed (Figs. 3H, I, 4A, D).

Forty-nine days after fertilization, two small ciliated lobes appeared in the basal region of the posterodorsal arms (Fig. 4A). By 53 days, these vibratile lobes had become very prominent, and it was apparent that they were not supported by skeletal elements (Fig. 4B). At 53 days, more than 50% of the larvae in all cultures were at the 10-arm stage, having posterolateral arms, posterodorsal vibratile lobes, and a posterior process. No additional arms developed after the 10-arm stage, though larvae continued to increase in size until the largest larva had attained a maximum length of 3071 μm 56 days after fertilization.

During the 10-arm stage, a ciliated ridge began to develop near each posterodorsal lobe. These two ridges ran in an anterior-posterior direction, parallel to the anterolateral rods on opposite sides of the larvae (Fig. 3H) and eventually joined to form a distinctive loop surrounding the region of the resorbing preoral and anterolateral arms (Fig. 4C). By the 75th day, the ciliated ridges were thick and heavily pigmented along their entire length (Fig. 4C) and were reflexed at the anterior end to form a loop that surrounded the region where the anterolateral and postoral arms had been (Fig. 4C). Also at this time, large brownish orange oil droplets were observed near the bases of the postoral, posterodorsal, and posterolateral arms.

Sixty-two days after fertilization, the posterior process of the largest larva attained its maximum length of 1224 μm ; the posterolateral arm was now 459 μm in length. The posterodorsal lobes continued to elongate until they attained a maximum size of 237.5 μm , 127 days after fertilization. Larvae began to decrease in overall length after the 75th day, but the postoral arms began to widen near the bases, until on the 134th day they had attained a maximum basal diameter of 384 μm (Fig. 4D).

A few larvae survived in culture for up to 151 days, though none metamorphosed. An echinus rudiment appeared in a single larva before the 77th day. This larva was isolated and exposed to deep-sea sediments in an unsuccessful attempt to induce metamorphosis. Many more individuals developed echinus rudiments between the 77th and 134th days. In most animals, anatomical details of the rudiment were difficult to visualize because the rudiment was opaque and was partially obscured by an equally opaque gut region (Fig. 4D, E). Nevertheless, podia were observed in the rudiments of some animals beginning on the 116th day, and adult spines were observed between 120 and 127 days after fertilization. As in many other species (Mortensen, 1921), the arms of late-stage larvae became smaller as the rudiment grew.

Larval skeleton

The postoral, posterodorsal, and posterior rods were all fenestrated and thorny (Fig. 4F), whereas the anterolateral

rods (Fig. 4F) and posterolateral rods (Figs. 3I, 4D) were simple, not fenestrated. The distal portions of the anterolateral and preoral rods were thorny and the proximal portions

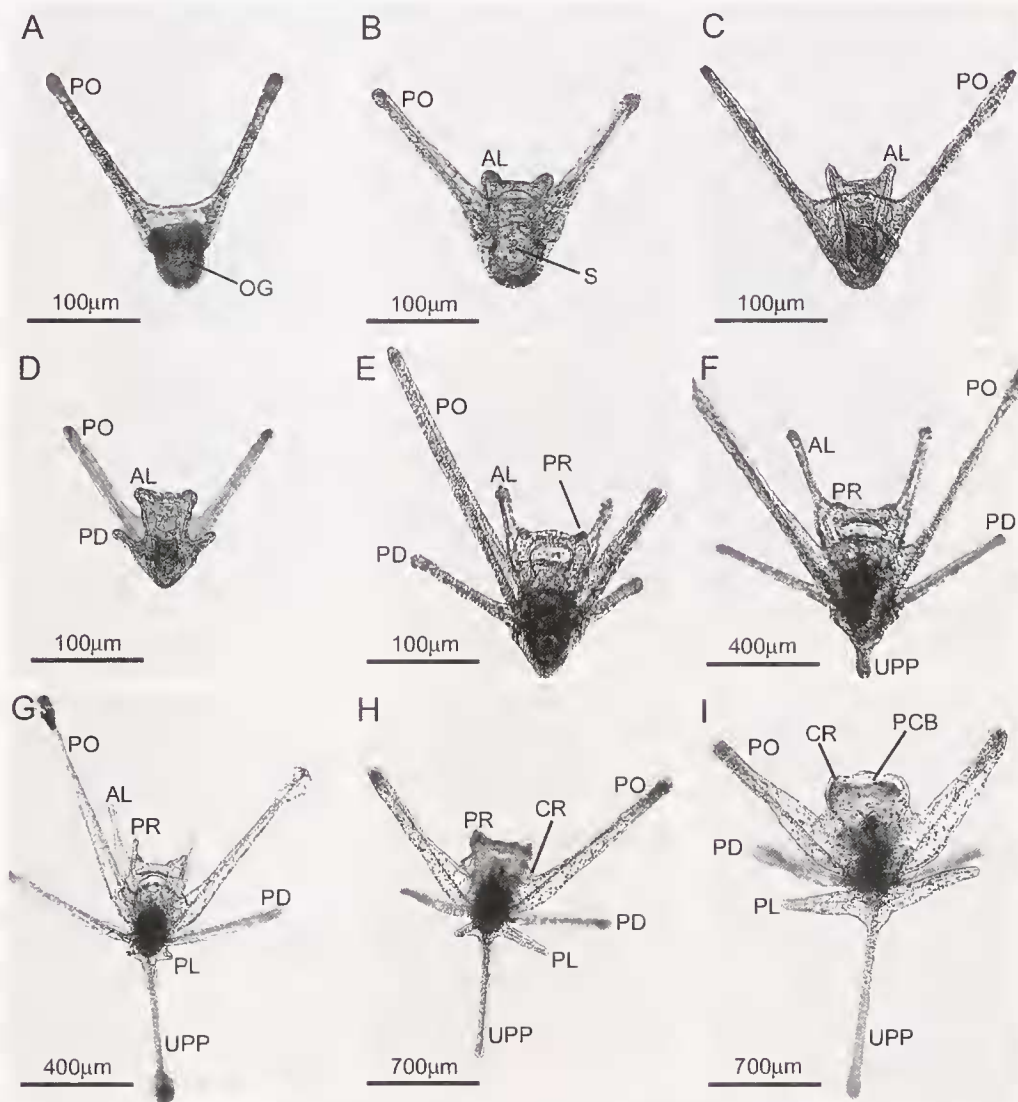


Figure 3. (A) Two-arm echinopluteus larva of *Aspidodiadema jacobyi* at 6 days, prior to the opening of the mouth, showing well-developed postoral arms (PO) and opaque gut region (OG). (B) Eleven-day-old larva in the early four-arm stage, viewed dorsally with newly formed anterolateral arms (AL). The stomach region (S) is becoming more visible. (C) Ventral view of a 15-day, 4-arm pluteus larva. Note that the anterolateral arms (AL) are supported by simple, unfenestrated rods. (D) Early 6-arm echinopluteus, 25 days old, viewed from the dorsal side with small posterodorsal arms (PD) developing. (E) Intermediate 6-arm echinopluteus stage viewed ventrally 28 days after fertilization, with well-developed posterodorsal arms (PD) and presumptive buds where the preoral arms (PR) will eventually develop. (F) Advanced 6-arm echinopluteus (ventral view), 32 days old, shortly after the first appearance of the unpaired posterior process (UPP). Preoral arms (PR) are still present only as tiny buds. (G) Ventral view of 39-day, early 10-arm larva showing well-developed unpaired posterior process (UPP) and preoral arms (PR). Posterolateral arms (PL) are just beginning to grow in this individual, but many in the same cultures were still at a late 8-arm stage. (H) Forty-two-day-old, 10-arm echinopluteus viewed ventrally just after the appearance of lateral ciliary ridges (CR). Posterolateral arms (PL) are growing asymmetrically and anterolateral arms have been resorbed. (I) Ten-arm larval stage (42 days old) after preoral and anterolateral arms have been resorbed. The posterior ciliary band (PCB) is visible as a dark region partially obscured by the transparent preoral lobe.

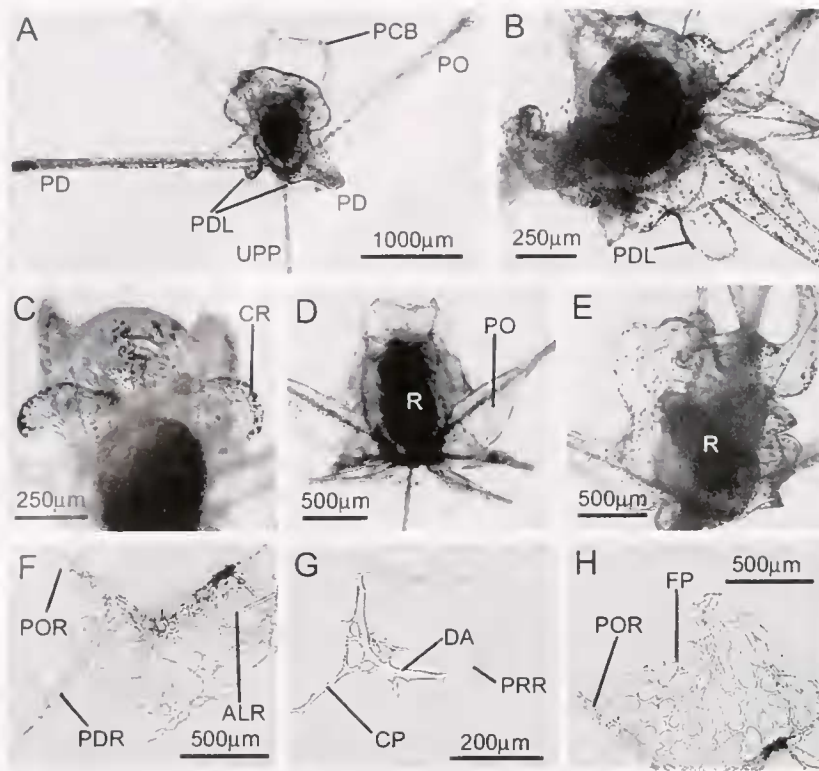


Figure 4. (A) Forty-nine-day-old echinopluteus larva of *Aspidodiadema jacobyi* in dorsal view. The posterodorsal lobes (PDL) have just appeared, and the posterior ciliary band is visible. PCB, posterior ciliary band; PO, postoral arm; PD, posterodorsal arm; UPP, unpaired posterior process. (B) Posterior view of a 53-day-old larva showing one of the large posterodorsal lobes (PDL). (C) Mid-anterior region of a 75-day-old echinopluteus, showing the lateral ciliated ridges (CR) now expanded into a large loop of cilia that surrounds the entire region of the resorbed pre-oral and anterolateral arms. (D) Ventral view of a 134-day-old larva with echinus rudiment (R) and very broad bases of the postoral arms (PO). (E) Echinopluteus larva 134 days old, showing echinus rudiment (R). (F) Posterior region of a late echinopluteus larva digested with sodium hypochlorite to reveal skeletal structures. Note that the postoral rods (POR) and posterodorsal rods (PDR) are fenestrated and tightly coupled with a basket-like stereom near their bases. The anterolateral rod (ALR) is simple. (G) Dorsal arch (DA) of an echinopluteus with preoral rods (PRR) originating on the two ends and a large central process (CP) in the middle. The central process is simple at the proximal end and fenestrated distally (barely visible at the far left-hand side of the micrograph). (H) Bases of the two postoral rods with their fenestrated plates of stereom (FP) fused together to create part of the posterior basket.

were smooth (Fig. 4G). The posterolateral rods were branched and extremely thorny. The dorsal arch was complex and slightly asymmetrical, having two large holes and one much smaller hole in the central region of the arch (Fig. 4G). A long central process, half of which was thorny and solid and the other half of which was thorny and fenestrated, projected from the base of the dorsal arch (Fig. 4G). Between the 42nd and 49th day after fertilization, the base of each postoral rod expanded into a dense network of stereom that eventually took the form of a highly fenestrated triangular plate (Fig. 4F). This plate fused with the corresponding plate at the base of the other postoral rod (Fig. 4F) to form a basket-like structure that filled the broad bases of the postoral arms (Fig. 4H, D). A slightly smaller fenestrated plate developed at the base of each posterodorsal rod (Fig. 4F). Together, the fenestrated plates originating from the

postoral rods and the posterodorsal rods formed a truncated pyramidal structure. The posterolateral rod and the fenestrated thorny rod (Fig. 3H) of the posterior process developed from the posterior transverse rod, which was situated dorsally.

Discussion

Early stages of embryology and larval development have been reported for a number of bathyal and abyssal invertebrates (Prouho, 1888; Mortensen, 1921; Young and Cameron, 1989; Young and Tyler, 1993; Young *et al.*, 1996b, c), but the complete larval development has been described for only a single eurybathic echinoid, *Cidaris cidaris* (Prouho, 1888). Thus, the present work is not only the first complete description of larval development in the family Aspidodia-

dematidae, but also the only complete developmental sequence known for any exclusively deep-sea invertebrate.

For the first time, we also have a direct estimate of the length of the pelagic larval period for a deep-sea animal. On the basis of these laboratory rearings, we may conclude that, given adequate food, larvae are capable of dispersing for at least 5 months. In the Tongue of the Ocean, Bahamas, we have measured current speeds as high as 18 cm/s at the depths where *Aspidodiadema jacobyi* lives (Young *et al.*, 1993). The current speed in this region averages about 5 cm/s and flows mostly in a single direction (Young *et al.*, 1993). Using these flow values for a rough eulerian calculation of dispersal distance, a larva could drift about 4.3 km per day, or 645 km in 5 months. The real dispersal distance, of course, will be influenced by the local current regimes, topographical features, and settlement opportunities that the larva encounters during the course of its drift.

Young *et al.* (1996a) showed that larvae of *A. jacobyi* cannot tolerate the high temperatures found in the upper 200 m of the water column, suggesting that dispersal occurs below the permanent thermocline and that larvae must feed on items sinking out of the euphotic zone. In an earlier study (Young *et al.*, 1989), it was noted that the larvae of *A. jacobyi* have an unusually long prefeeding period, and that they can survive on maternal reserves for up to 2 months. It was hypothesized that this attribute may confer nutritional flexibility on these larvae, enabling them to disperse and survive in deep oligotrophic waters where food items are scarce and patchy (Young *et al.*, 1989). In the present study, we detected no additional morphological attributes that can be interpreted as special adaptations for deep-sea dispersal. Complex ciliation and long arms presumably keep these large larvae suspended in the water column, but similar complexity is found in late-stage larvae of many shallow-water echinoids (Mortensen, 1921; Emler, 1988b; Pearse and Cameron, 1991). The large posterior process on the larva of *A. jacobyi* lowers the center of mass, presumably facilitating upright orientation (Pennington and Strathmann, 1990; Young, 1995), but this is a feature also found in shallow-water spatangoid larvae (Mortensen, 1921).

The developmental rate of *A. jacobyi* to the 2-arm stage (6 days) was similar to that reported by Young *et al.* (1989), but after the 2-arm stage, differences were observed between the two studies. The mouth opened 11 days after fertilization in the present study and between 18 and 21 days after fertilization in the study by Young *et al.* (1989). Fully developed 4-arm *A. jacobyi* larvae were observed in cultures 15 days after fertilization in the present study, but not until 24 days after fertilization by Young *et al.* (1989). Diet could explain differences in development after the onset of feeding, but not differences in the rate at which the mouth developed. The differences between these two studies could be attributed to genetics, egg quality (Emler *et al.*, 1987; George *et al.*, 1990; George, 1996) or culture conditions.

Culture conditions might also explain why the preoral and anterolateral arms (and occasionally other arms: Fig. 4A) were resorbed in most individuals shortly after the onset of the 10-arm larval stage. On the other hand, many of these larvae survived for an additional 2 months or more and some developed rudiments, suggesting that they were not unhealthy. Resorption of some arms as others develop appears to be normal in certain species of echinoids (Mortensen, 1921; Pearse and Cameron, 1991).

Mortensen (1921, 1931) described the morphology of echinoderm larvae in minute detail with the expectation that larval characters would be keys to the unraveling of phylogenetic relationships within the phylum. Since that time, larval characters have been widely used for interpreting life-history evolution (reviewed by Havenhand, 1995; Wray, 1995), and for resolving phylogenetic issues at higher taxonomic levels (*e.g.*, Hadzi, 1963; Strathmann, 1988; Nielsen, 1995). However, recent analyses that combine genetic data with adult and larval character sets have shown that larval characters often evolved independently of adult body form (Smith and Littlewood, 1997) and that larval characters are more prone to homoplasy than adult characters (Smith *et al.*, 1995, 1996). Wray (1992) pointed out many similarities among larvae from groups as unrelated as ophiuroids and diadematoids, and as spatangoids and phymostomatoids. These commonalities are problematic for interpreting phylogeny; indeed, both Strathmann (1988) and Raff *et al.* (1988) have recognized that any phylogeny based on larval characters must accept a number of remarkable convergences. Despite the problems of interpreting larval characters from a phylogenetic standpoint, parsimony demands that we be very careful before concluding that an attribute has evolved independently in multiple clades.

The order Diadematoida is currently composed of three families: Diadematidae, Lissodiadematidae, and Aspidodiadematidae (Smith, 1984). The fully developed larva of *A. jacobyi* differs significantly from the known diadematid larvae in its extraordinary complexity. Whereas larvae of *A. jacobyi* have five pairs of larval arms and a posterior process, larvae in the family Diadematidae have only one pair of larval arms (the postorals) and no posterior process (Mortensen, 1921, 1931). Although the family Aspidodiadematidae is found exclusively in deep water and the other two families are restricted to shallow waters, the known larvae of diadematoids do share a number of common features. Like *Aspidodiadema*, all known larvae in the Family Diadematidae (*e.g.*, *Diadema antillarum*, *Astropyga pulvinata*, and *Diadema setosum*; Mortensen, 1921, 1931) have very long, thorny, and fenestrated postoral rods; a body skeleton in the shape of a 4-sided truncated pyramid; and a posterior transverse rod, which in some species takes the form of a fenestrated triangular plate during advanced stages. Most significantly, Mortensen (1921) reported that the larva of "*Echinopluteus transversus*" e and f (later

shown to be *Diadema setosum*) has a very short, fenestrated, and unpaired ossicle immediately below the posterior transverse rod. He expressed the opinion (Mortensen, 1921, page 90) that although this structure bears a superficial resemblance to the posterior process of spatangoid larvae, the structures are not homologous. Whereas the spatangoid posterior process is an outgrowth of the posterior transverse rod, that of the diadematid larva is not connected to the rest of the skeleton.

Larvae of *Aspidodiadema jacobyi* have several features in common with larvae of the phymostomatoids *Arbacia lixula* (Fenaux, 1969) and *A. punctulata* (Garman and Colton, 1883). Like *A. jacobyi*, these species possess very long postoral and posterodorsal arms, swept-back posterolateral arms, a pair of vibratile lobes, and fenestrated triangles of stereom that form at the base of the postoral and posterodorsal rods before metamorphosis. The *Arbacia* species, like *A. jacobyi*, also develop parallel ciliated ridges that originate in the region of the posterodorsal arms and run parallel to the anterolateral rods along the midsection of the larva (Fenaux, 1969). However, these ridges terminate with the formation of the sixth pair of larval arms (anterodorsal arms) in the phymostomatoids, not with a ciliary ring around the base of the preoral and anterolateral arm region as in *A. jacobyi*.

Because of its posterolateral arms and unpaired posterior process, larvae of *A. jacobyi* bear a striking resemblance to the characteristic larval form of irregular urchins in the order Spatangoida (Mortensen, 1921; Rees, 1953). The fenestrated basket structure of *Aspidodiadema* larvae is also very similar to the basket that characterizes the Clypeasteroids, another major clade of irregular urchins (Pearse and Cameron, 1991). Larvae with posterior processes have been hitherto unknown outside the spatangoids, and all authors to date have considered this character to be absolutely diagnostic of this order (Mortensen, 1921; Pearse and Cameron, 1991). Other features shared by these two groups include long postoral and posterodorsal arms and well-developed anterolateral arms. However, spatangoid larvae have six pairs of arms, whereas *Aspidodiadema* larvae have only five. The loss of arms is easy to explain in the evolution of larval forms, but the independent acquisition of a structure as distinctive as a fenestrated, unpaired posterior process in two different clades seems improbable.

Emler (1988a) used crystal orientations in the apical plates to predict that all larvae in the family Aspidodiadematidae should have at least two pairs of larval arms (postoral and posterodorsal arms) and less widely spread larval arms than the Diadematidae and Lissodiadematidae, both of which should have only widely spread postoral arms. On the basis of this prediction, Emler (1988a) suggested that the family Aspidodiadematidae must be a sister group to the families Diadematidae, Lissodiadematidae, and Micropygidae, all of which have only a single pair of larval arms (Fig. 5). Our findings lend strong support to Emler's recommen-

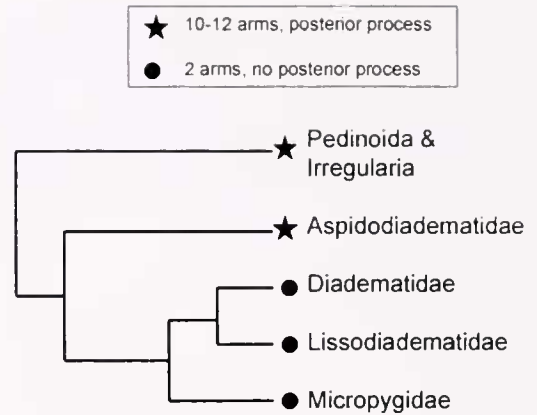


Figure 5. Phylogenetic tree suggested by and redrawn from Emler (1988a). This tree organizes the Diadematida and related euechinoids in such a way as to preserve the important relationships of adult characters (Jensen, 1981) while requiring that the 2-arm echinopluteus evolve only once. The larval form of *Aspidodiadema* fits perfectly into this scheme, with no revisions required to satisfy the demands of parsimony.

dation. The larval form of *Aspidodiadema* is interpreted parsimoniously only if the Aspidodiadematidae are classified as a separate order outside the Diadematoida or as a sister group to the other families within the order. Indeed, the remarkable similarities between *A. jacobyi* larvae and those of the spatangoids and clypeasteroids suggest that the Aspidodiadematidae may be near the stem group that separated regular and irregular euechinoids.

Acknowledgments

We thank Mary Clark, who supplied us with algae; Paul Tyler, who assisted at sea and helped spawn the urchins; and Tracy Griffin, who assisted in the preparation of figures. Andrew Smith and Richard Mooi advised us in matters of phylogeny, though the opinions we express herein are entirely ours. This work was funded by the National Science Foundation (OCE-9116560 and OCE-9633784) and by a Harbor Branch postdoctoral fellowship to Sophie George. HBOI contribution number 1339.

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